Lufenuron can be transferred by gravid Aedes aegypti females to breeding sites and can affect its fertility, fecundity and blood intake capacity

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Research

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Abstract

Background: Aedes aegypti (L.) is the main vector of dengue, yellow fever, Zika and chikungunya viruses. A new method for controlling this mosquito has been developed based on the possibility that wild adult mosquitoes exposed to artificial resting sites contaminated with a larvicide, can disseminate it to larval breeding sites, is named “auto-dissemination”. The present study was undertaken to evaluate if a chitin synthesis inhibitor like lufenuron can by disseminated to larval breeding sites and prevent adult emergence and also if forced contact of Ae. aegypti females with treated surfaces can affect its fertility, fecundity and blood intake capacity. Methods: Larval susceptibility to lufenuron was measured through EI 50 and EI 90 . On the other hand gravid females were exposed by tarsal contact to lufenuron-treated papers, we used the WHO susceptibility test kit tube to line the papers, and 1, 3 or 5 females for the transference. We also evaluated if the exposure of mosquito females to lufenuron-treated papers (0.4 and 1 mg a.i./cm 2) has an effect on their fertility, fecundity or in the ability to feed on blood. In each assay 12-15 mosquito females were exposed to lufenuron for 1 hour; 24 h before (before blood meal - BBM) or 24 h after a blood meal (ABM). Results: Lufenuron proved to be very active against Ae. aegypti larvae with an EI 50 of 0.164 ppb and EI 90 of 0.81 ppb. We also found that lufenuron can be transferred by females from treated surfaces to clean containers causing the inhibition of emergence of the larvae (between 30 and 50%). This effect was dependent on the concentration applied on the paper and also the number of females added to each cage. Conclusions: This paper introduces an innovation by first exploring the possibility that an IGR belonging to the group of benzoylphenyl ureas, such as lufenuron, can be transferred by gravid females to breeding sites and that at the same time can have an effect on fertility, fecundity and blood intake capacity of adult mosquitoes. Keywords: Aedes aegypti , lufenuron, auto-disemination, fertility, fecundity.

Background

Aedes aegypti (L.) is the main vector of dengue, yellow fever, Zika and chikungunya viruses in many parts of the world affecting millions of people worldwide each year. The most commonly utilized strategy to reduce *Ae. aegypti* densities is aimed at the larval stages (removal of breeding sites, larvicide and community education) to reduce the population of new adults. Also, adult control using spatial sprays with adulticides is recommended when dengue outbreaks occurs [1]. Because adult emergence from container habitats is continuous, conventional adult insecticides spraying generally achieves inadequate and merely transient control [2-6].

The use of larvicides in containers that can result as potential breeding places and cannot be eliminated is the main alternative in control programs, but this only targets an unknown percentage of the overall aquatic habitat. Application of larvicides to containers used as oviposition sites requires a house-to-house search to find and treat containers, which may not be feasible in large communities. In addition, treating larval production sites has produced insecticide resistant populations especially to the principal larvicide used the last years, the organophosphorus temephos; pointing out the need of new larvicides for mosquito control [7-11].

During the past two decades, considerable progress has been made in the development of natural and synthetic compounds that are capable of interfering with the growth, development and reproduction processes of insects [12]. These substances are classified as insect growth regulators (IGRs) and compared with other insecticides, are safer for the environment and non-target organisms, including mammals, at the recommended doses [13-15] There are three major groups of IGRs: the juvenile hormone analogues, the ecdysone agonists and the chitin synthesis inhibitors [12, 16]. A common property of this last group of IGRs, also called benzoylureas (BPU), is that they result in abortive molting and egg hatching as a consequence of chitin synthesis inhibition in the course of cuticle formation. The first chitin synthesis inhibitor introduced in markets was diflubenzuron [17]. This IGR was used successfully against various pest insects, including mosquitoes [14, 18, 19]. Among the most successful benzoylurea compounds next to diflubenzuron are triflumuron, hexaflumuron, lufenuron and novaluron. Lufenuron is one of the most newly introduced synthetic benzoylurea (CibaGeigy in 1998) used for the control of lepidopteran and coleopteran larvae. It is a compound found to be nontoxic to mammals and other vertebrates at the doses required against insects. Furthermore, it has been reported that lufenuron is suitable for integrated pest management programs because of its long residual action and safety to adult beneficial insects, mites and spiders [20]. Although several reports describe the effects of different benzoylureas, against disease vectors [21–25], there is only one study that evaluates the toxicity of lufenuron on *Ae aegypti* larvae, nothing is known about its effect in the biology and reproductive fitness of the adult.

Aedes aegypti is a diurnal species that displays skip-oviposition behavior, i.e. lays small numbers of eggs in multiple sites [26]. These sites are often small and difficult to locate, which makes effective larvae elimination difficult. A novel method of control for this mosquito species was suggested from laboratory research results reported by Itoh and colleagues [27]. They found that bloodfed females of *Ae. aegypti* that had been forced into contact with surfaces treated with the IGR, pyriproxyfen (a juvenile hormone analogue), transported sufficient amounts to disrupt larval development in untreated oviposition sites. This approach, named “auto-dissemination”, is based on the possibility that wild adult mosquitoes exposed to artificial resting sites contaminated with pyriproxyfen, can disseminate insecticide to larval breeding sites, thus preventing adult emergence. Extraordinarily low doses of pyriproxyfen are needed to interfere with the metamorphosis of juvenile stages [28], and/or to cause morphological and functional aberrations in emerging adults, such as decreased fertility [29-31].

Since the work of Itoh and colleagues [27] a lot of evidence has been collected in laboratory and field works that shows that mosquito females either forced to walk on pyriproxyfen-treated surface or topically contaminated can contaminate larval sites and significantly inhibit adult emergence [28, 32-35]. However, it has never been evaluated if an IGR belonging to chitin synthesis inhibitors group can be disseminated, which doses are needed on a contaminated surface to have an effect in untreated oviposition sites and if they can affect the adult fecundity and fertility.

The present study was undertaken to evaluate if a BPU like lufenuron can by disseminated to larval breeding sites and prevent adult emergence and also if forced contact of *Ae. aegypti* females with treated

surfaces can affect its fertility, fecundity and blood intake capacity.

Methods

Mosquitoes

A susceptible strain of *Ae. aegypti* (CIPEIN) was used. This strain was originated from a Rockefeller strain from Venezuela and had been kept in the laboratory since 1996, reared at 26 ± 2ºC under a 12:12 h light: dark photoperiod. This colony is maintained free of exposure to pathogens, insecticides or repellents. Eggs were collected over wet cotton then dehydrated at room temperature and stored at least 30 days. They were rehydrated in dechlorinated water (about 500 eggs per 2 liters of water) and 24 h after rehydration, first instar larvae were observed. Larvae were fed on a mixture of rabbit pellet and yeast. For this study, late third-instar or early fourth-instar larvae were used, and 5-7 days old adults females.

Chemicals

Lufenuron (N-[(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)carbamoyl]-2,6-difluorobenzamide), (technical grade 97.8%; Zhejiang Sega Science and Technology Co., Ltd, China) was used.

Silicone oil Dow Corning 556 was purchased from Daltosur SRL, Buenos Aires, Argentina.

All solvents used (acetone) were analytical grade.

Determination of larval susceptibility to lufenuron

The larvicidal bioassay was completed following the protocol by Bisset and colleagues [36]. One milliliter of the insecticide solution to be assayed was added to 224 mL of water in a 500 mL plastic container, and then was shaken lightly to ensure a homogeneous test solution. Then, 25 mL of water with 20 late third or early fourth instar *Ae. aegypti* larvae were added to the container. Five different concentrations of lufenuron were tested, ranged from 0.016 to 1 mg/L (ppb). One milliliter of solvent (acetone) was added to other cup and used as a control. Each concentration, including the control, was replicated five times. Cups containing the treated larvae were placed in a regulated chamber (26 ± 2ºC, 60-70% RH and 12:12 h photoperiod) with 100 mg of food mixture supplied once.

Bioassays were monitored for several days, until all insects either died or reached adulthood. Adult emergence inhibition (EI) data were registered as soon as all control group specimens emerged. The results were used to calculate the EI_{50} and EI_{90} values using the Probit method [37]. These values correspond to the lufenuron doses necessary to inhibit adult development of 50 and 90% of the specimens treated, respectively.

Transfer of lufenuron by treated females to clean containers with larvae

Gravid females were exposed by tarsal contact to lufenuron-treated papers to determine if mosquitoes, after walking on the treated paper, could transport sufficient amounts to disrupt the development of larvae held in containers with water.

Methods modified from Itoh and colleagues [27] were used. Technical grade lufenuron was diluted with a 9:1 mixture of acetone : silicone oil and applied to Whatman® N°1 filter papers (12 x 15 cm) at an application rate of 0.2, 0.4 and 1 mg active ingredient per cm^2 (mg a.i./cm^2). These doses were chosen based on previous work done with pyriproxyfen [32] where similar doses were used; also knowing that lufenuron has a higher $EI_{50}/_{90}$ and therefore is less effective, a slightly higher dose was also added (1 mg/cm^2). After acetone–silicone oil solutions of lufenuron were applied, paper discs were air dried in darkness for 1 h and used to line the inside wall of a WHO susceptibility test kit tube and held in place by a wire ring.

Fifteen females, 5 to 7 days old, were confined to the test kit for 1h. They had been blood fed 3 days before and the day of the treatment. After exposure to treated paper 1, 3, or 5 treated females were released into a mosquito cardboard cage of 5 kg (13 cm height x 21.5 cm diameter) with gauze as lid. A plastic container (8 cm top diameter, 7 cm bottom diameter x 5 cm high) containing 20 late 3^{rd}/ early 4^{th} instar larvae in 100 ml of water and lined with filter paper was added to the cage. The females were allowed to lay eggs for five days on the filter paper. The filter paper was then removed for observation of egg deposition. If any of the females in the cages retained eggs the assay was discarded. Larvae in the plastic container were fed daily and reared at 26 ± 2°C until adults emerged or all individuals die. Adult emergence inhibition (EI) data were calculated as described in the larvicidal bioassay.

Each combination of lufenuron dose and number of IGR-exposed females was replicated 5 times. Control papers were treated with 1 ml of the acetone–oil mixture, and used as described for IGR-treated papers.

Mosquito cardboard cages were discarded once finished the assay and new ones were used for the next replicates.

Effect of lufenuron on female fertility and fecundity

As described before females were exposed by tarsal contact to lufenuron-treated papers (0.4 and 1 mg a.i./cm^2) to determine if it has an effect on their fecundity (increase or decrease of the number of eggs laid), fertility (reduction of hatchability or viability of eggs) or in the ability to feed on blood.

In each assay 12-15 mosquito females were exposed to lufenuron for 1 hour; 24 h before (before blood meal - BBM) or 24 h after a blood meal (ABM). Then females were released into a mosquito cardboard cage in which a container with 100 ml of water lined with Whatman® N°1 filter paper (20 x 6 cm) as oviposition substrate was placed.

The females were allowed to lay eggs for 5 days and the number of eggs laid per female was counted, both on the filter paper and in the water. Females were removed, dissected and the assay was discarded if

retained eggs were found. The filter papers were left to dry and stored in sealed, plastic bags for 15 days to allow eggs to complete embryogenesis. After this period a known number of eggs were placed in water to evaluate egg hatch and therefore, fecundity. As *Ae. aegypti* eggs usually hatch erratically or asynchronous depending on the environmental conditions [38], the number of larvae was counted after two, four and seven days.

For those females that were first exposed to lufenuron and then fed (BBM), we also evaluate mortality 24 h after exposure and the percentage of females that fed on blood.

Statistical analysis.

In the determination of larval susceptibility to lufenuron bioassays, data was subjected to log-dose, probit-mortality analysis (PoloPlus 2.0 software LeOra Software Company, Petaluma, CA). Prior to these analyses, the percentage inhibition of emergence of adults in treated container was corrected for mortality in control containers [39].

Results of forced-contact experiments were analyzed by MANOVA (Sigmaplot 11.0, Systat Software Inc., San Jose, CA) to determine if EI% varied significantly between trials, the number of females per bioassay cage or application rates. To compare the number of eggs/female, the percentage of hatched eggs and the females that feed on blood after exposure to lufenuron between the treated and control group a Student t test was used or a Wilcoxon test (Mann-Whitney) when the assumption of normality was not met. The level of significance was set at $p \leq 0.05$ (Sigmaplot 11.0, Systat Software Inc., San Jose, CA).

Results

Transfer of lufenuron by treated females to clean containers with larvae

Lufenuron proved to be very active against *Ae. aegypti* larvae with an EI_{50} of 0.164 ppb (CI_{95} 0.039-0.486 ppb) and EI_{90} of 0.81 ppb (CI_{95} 0.32-33.3 ppb) (Table 1).

We also found that lufenuron can be transferred by females from treated surfaces to clean containers causing the inhibition of emergence of the larvae. This effect was dependent on the concentration applied on the paper ($F = 4.92$, $df = 2$, $P = 0.013$) and also the number of females ($F = 4.75$, $df = 2$, $P = 0.015$) added to each cage. When the lowest dose of lufenuron was applied (0.2 mg/cm^2) there were no differences in the number of females used with a mean EI% between 17 and 30% (Fig. 1).

On the other hand, no differences in EI% were found between the doses applied to the paper when only one female was used to transfer lufenuron, indicating that there is a maximum amount that a female can transfer regardless of the dose with which she comes in contact (17, 23 and 30% EI for doses of 0.2, 0.4 and 1 mg/cm^2 respectively). At the highest doses (0.4 and 1 mg/cm^2) no differences were found between using 3 or 5 females. A slight tendency of higher larvae mortality values is observed in the dose of 1 mg/cm^2 although on average they do not present significant differences with the dose of 0.4 mg /cm^2.

Effect of lufenuron on female fertility and fecundity

The effect of lufenuron on the immature stages of insects is well known, however little is known about its direct effect on adults. In this trial the effect of lufenuron on fertility, fecundity and blood intake capacity of adult *Ae. aegypti* females was evaluated.

When females were exposed to lufenuron 24 h after blood meal (ABM) no differences were found in the number of eggs laid per female between the treated (0.4 or 1 mg/cm^2) or the control group (Table 2). No significant differences were found in the hatching percentage between the control group (82.7%) and the females that were exposed to a dose of 0.4 mg/cm^2 (78.5%). However, significant differences were found in the group in which females contacted a surface with 1 mg/cm^2 of lufenuron (83.1% in control group vs. 44.8% in treated, T = 3.42, df = 4, P = 0.026).

When the females were first exposed to lufenuron and fed after 24 h of this exposure (BBM) no differences were found in the number of eggs laid per female between the treated (0.4 or 1 mg/cm^2) or the control group (Table 2). Also no significant differences were found in the hatching percentage between the control group (89.8%) and the females that were exposed to a dose of 0.4 mg/cm^2 (79.5%). However, significant differences were found in the group in which females contacted a surface with 1 mg/cm^2 of lufenuron (88.3% in the control group vs. 46.6% in the treated one; W = 26, P = 0.029).

Also for the BBM treatment, no differences were found in the percentage of mortality after 24 h between the females who had contacted a lufenuron surface before blood meal in both the 0.4 mg/cm^2 (5.1%) or 1 mg/cm^2 (7%) dose and the control females (3%). Regarding the ability to blood feed of those females who had previously contacted a lufenuron-treated surface, we found that 63.6% of the females who contacted a surface with 0.4 mg/cm^2 fed while in the control group this percentage was 89.2%, although the difference between groups was greater than 20%, it was not statistically significant.

On the other hand, 56.2% of the females who contacted a surface with 1 mg/cm^2 of lufenuron fed while in the control group this percentage was 97%, with significant differences between both values (W = 37.5, P= 0.039).

Discussion

Future challenges in integrated pest management require the development of selective and environmentally safe pesticides along with new strategies to apply them. The more specifically these insecticides act, and the less are their adverse side effects on beneficial insects and the environment, the more they are appropriated to control arthropod pests. Insecticides with novel modes of action such as chitin synthesis inhibitors disrupt cuticle formation [40]; suppression of chitin deposition in treated insect often causes high mortality during molting [41].

The survivorship of mosquitoes is very important for production of progeny, development, and transmission of pathogens among the hosts. Several studies reported the effects of sublethal exposure of IGRs on survival, fecundity, fertility and blood intake capacity of mosquito females [28-31, 42-45]. However, very few studies have evaluated the direct effect of IGRs, mainly pyriproxyfen, on adult mosquitoes [46-49].

Among the new strategies that are trying to be implemented to control vector mosquitoes is the approach called "auto-dissemination", based on the possibility that wild adult mosquitoes exposed to artificial resting sites contaminated with IGRs (so far pyriproxyfen, a juvenile hormone analogue), can disseminate insecticide to larval breeding sites, thus preventing adult emergence [28, 30, 32, 33]. This strategy is facilitated by the oviposition behaviour of *Ae. aegypti*, that typically distribute the eggs from a single gonothrophic cycle among several temporary sites. The "auto-dissemination" approach can be proposed as a 'pull' (i.e attraction of wild mosquitoes to contaminated sites) and 'push' (i.e. dispersal of contaminated mosquitoes and dissemination of IGR to larval habitats) control strategy with the potential to target the myriad of cryptic larval breeding sites that cannot be reached by traditional larvicidal applications.

The objective of our work was to evaluate for the first time if an IGR belonging to the chitin synthesis inhibitors group can be transferred by mosquito females who contact a treated surface to larval breeding sites. On the other hand, it was also studied whether the contact with a lufenuron-treated surface can have effects on female's fertility, fecundity and their blood intake capacity.

In our work, lufenuron proved to be highly effective on *Ae. aegypti* larvae with an EI_{50} of 0.164 ppb and an EI_{90} of 0.81 ppb. These values were significantly lower than those found by Salokhe and colleagues [50], they obtained a value of 6 ppb; although they do not specify if used lufenuron technical grade or a formulation, so we cannot make a direct comparison with our results. The values obtained in our work are similar to those obtained for other IGRs of this group such as triflumuron [24] with a value of EI_{50} and EI_{90} of 0.8 and 1.8 ppb, respectively. Also for diflubenzuron a value of 0.5 ppb and 3.5 ppb was obtained for the EI_{50} and EI_{90} [44]. Although the EI_{50} values for pyriproxyfen, the IGR used in the "auto-dissemination" assays, are approximately 10 times smaller (EI_{50} = 0.011 ppb, [27]), we believe that the values obtained for lufenuron indicate that it would be a good candidate to explore in future field trials.

Regarding the "auto-dissemination" or horizontal transfer of lufenuron, our work shows that mosquito females contaminated with lufenuron can transfer enough material to water containers to exert a significant lethal effect on larvae developing there. This effect was dependent on the concentration applied on the paper and also the number of females added to each cage. This is the first work where it is demonstrated that an IGR of the BPU group can be transferred to larval breeding sites by mosquito females. On the other hand there is numerous evidence of this phenomenon for pyriproxyfen [20, 30, 31] were mortality is almost exclusively limited to the pupal stage [27]. However, the use of IGR of the BPU group could mean an advantage since mortality would occur earlier in development, that is, between larval molts or molting from larvae to pupa.

The doses of lufenuron used in our work were slightly higher than those used in other works with pyriproxyfen. This is due on one hand to the difference in effectiveness between both active ingredients (measured as EI_{50}), and on the other hand because the surfaces used in the works using pyriproxyfen were different from the paper used in our study. For example, in the work of Itoh and colleagues [27] a film of polyethylene terephthalat was used while Chism and Apperson [32] used seed germination papers. These surfaces could allow a greater bioavailability of the IGR therefore it would be interesting to repeat our work using different surfaces and evaluate if this could have any differential effect.

Itoh and colleagues [27] reported equivalent effects were achieved using 1, 3 or 5 *Ae. aegypti* females, however Chism and Apperson [32], using *Ae. albopictus*, found that percentage mortality was significantly lower at a density of one female per cage and increased to higher and equivalent levels of mortality at 3 and 5 females, they argue this difference to a variation in sensitivity between both species. In the case of our work, the results were similar to those of Chism's work, probably because as lufenuron is less potent as a larvicide than pyrirproxyfen, the amount of females that are involved in the transfer of the IGR is important.

In the last part of our work the effect of lufenuron on fertility, fecundity and blood intake capacity of adult *Ae. aegypti* females directly exposed to a surface treated with lufenuron was evaluated. When females were exposed to lufenuron 24 hours after blood meal (ABM) a reduction in the hatching percentage close to 40% was observed of those females that had contacted a surface with 1 mg/cm^2. Also, when the contact with the surface treated with the IGR was before blood feeding (BBM), a 40% reduction in egg hatching was observed at the dose of 1 mg/cm^2. This would indicate that the moment at which contact with lufenuron occurs (before or after blood feeding) is not decisive in the effect on fertility and fecundity but the dose used does; since the same results were obtained with both ABM and BBM regimes. On the other hand, the studies developed with pyriproxyfen indicate that the moment of contact with this IGR is decisive for its effect on the fertility and fecundity of the adult's mosquitoes. Aiku and colleagues [51] found significant effect on egg hatching when *An. stephensi* females were exposed to bednet treated with 2% pyriproxyfen 24 hours after blood feeding. Also Gaugler and colleagues [34] found the same results with *Ae. albopictus*, suggesting that mosquitoes are most susceptible when bloodfed one day prior to pyriproxyfen exposure and therefore exposed while developing their eggs. Itoh and colleagues [27] reported that tarsal contact of *Ae. aegypti* with 0.1 mg/cm^2 pyriproxyfen before a bloodmeal induced a large decrease in the number of eggs hatch compared with contact after a bloodmeal.

Finally, our work shows a reduction in the percentage of females that fed on blood for the dose of 1 mg/cm^2 of lufenuron, from 97% in the control group to less than 60% in the treated one. However, there was no effect of previous exposure to lufenuron on female mortality. There are no other works that evaluate the direct effect of IGRs on the blood intake capacity of mosquito females. Only the work of Vasuki [45] found that *Ae. aegypti* larvae and pupae exposed to sublethal doses of the IGR hexaflumuron significantly reduced the quantity of blood ingested as adult females with a corresponding reduction in egg laying.

Conclusion

During the past decades, a larger number of chemically unrelated insecticidal compounds have been developed and commercialized that interfere with chitin synthesis, which is essential to reproduction, growth, and development of insects [52]. This paper introduces an innovation by first exploring the possibility that an IGR belonging to the group of BPUs, such as lufenuron, can be transferred by gravid females to breeding sites and that at the same time can have an effect on fertility, fecundity and blood intake capacity of adult mosquitoes. Although lufenuron is effective against many insects, the proposed approach targets container breeding species with such tiny amounts of compound disseminated exclusively to their breeding sites, that impacts on non-target species are likely to be minimal. In the future it might be interesting to explore lufenuron ability to replicate the results found here in field conditions.

Abbreviations

BPU: Benzoylureas

EI: Adult emergence inhibition

EI%: Adult emergence inhibition percentage

EI_{50}: Adult emergence inhibition 50%

EI_{90}: Adult emergence inhibition 90%

CI_{95}: Confidence limits 95%

ABM: After blood meal

BBM: Before blood meal

Declarations

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics approval

No human participants, human data or human tissue were used in the present study. Insects were fed on pigeon blood once per week according to a protocol approved by the Institutional Animal Care and Use Committee of CIPEIN (IACUC/CICUAL 1531/13).

Acknowledgment

Not applicable

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PVG and LH designed, performed the study, and analysis of the data. PVG and LH wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1.

Title: Toxicity of lufenuron to laboratory-reared late 3rd and early 4th instar *Ae. aegypti* exposed continuously in the laboratory.

Specie	*N*	EI_{50} (ppb)	CI_{95} (95% fiducial limits)	EI_{90} (ppb)	CI_{95} (95% fiducial limits)	Slope	SE
Ae. aegypti	600	0.164	0.039-0.486	0.81	0.32-33.3	1.85	0.17

Legend: EI_{50} and EI_{90} (CI_{95} limits) for lufenuron expressed in mg/L (ppb). Abbreviations: $EI_{50\text{-}90}$, Adult emergence inhibition 50-90%; CI_{95}, 95% confidence interval.

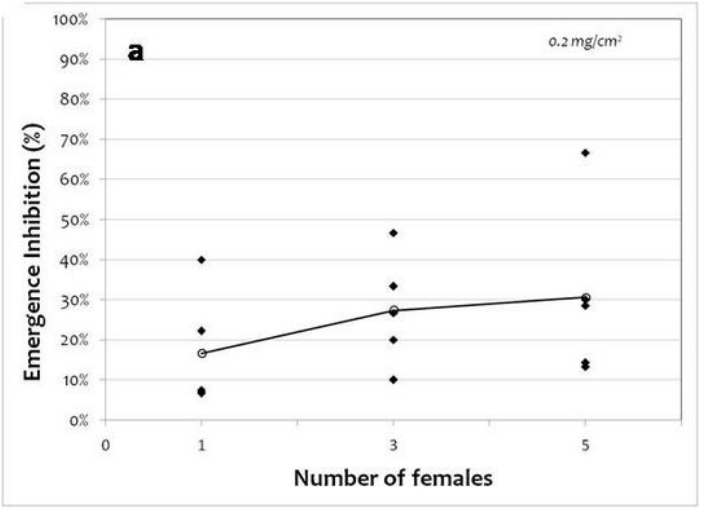
Table 2.

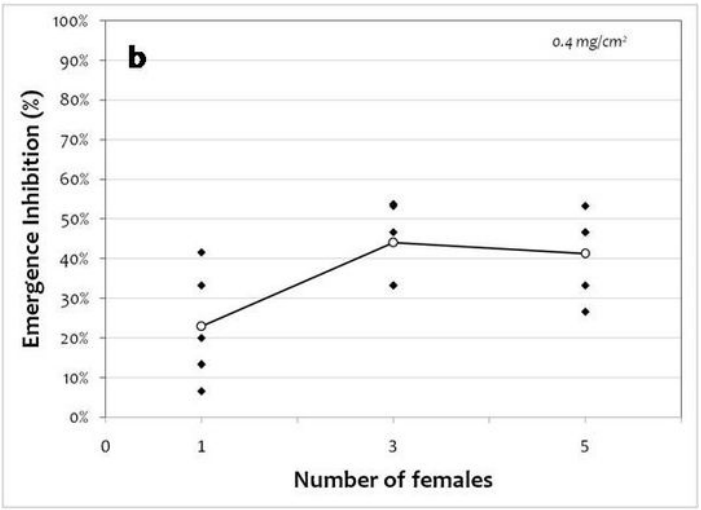
Title: Effects of IGRs lufenuron on egg production, hatchability of eggs, mortality and blood fed of *Ae. aegypti* females.

		Number eggs / female (±SE)		Hatching (%) (±SE)		Mortality (%) (±SE)		Females fed (%) (±SE)	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
BBM (Exposition to lufenuron Before Blood Meal)	0.4 mg/cm^2	54.2 ± 4.4	48.2 ± 2.5	89.9 ± 4.4	79.5 ± 7.5	2.9 ± 1.8	5.1 ± 2.1	89.2 ± 6.7	63.6 ± 16.1
	1 mg/cm^2	46.9 ± 3.8	38.4 ± 10.9	88.3 ± 4.6	46.6 ± 20*	2.9 ± 1.8	7.0 ± 5.3	97.0 ± 2.0	56.2 ± 19.9*
ABM (Exposition to lufenuron After Blood Meal)	0.4 mg/cm^2	49.6 ± 6.5	49.2 ± 3.3	82.7 ± 2.1	78.5 ± 4.5	----	----	----	----
	1 mg/cm^2	54.8 ± 7.6	47.7 ± 4.2	83.1 ± 1.1	44.8 ± 11.2*	----	----	----	----

Legend: *significant differences between treated and control group ($P < 0.05$).
Abbreviations: *ABM:* After blood meal; *BBM:* Before blood meal; SE: standard error.

Figures





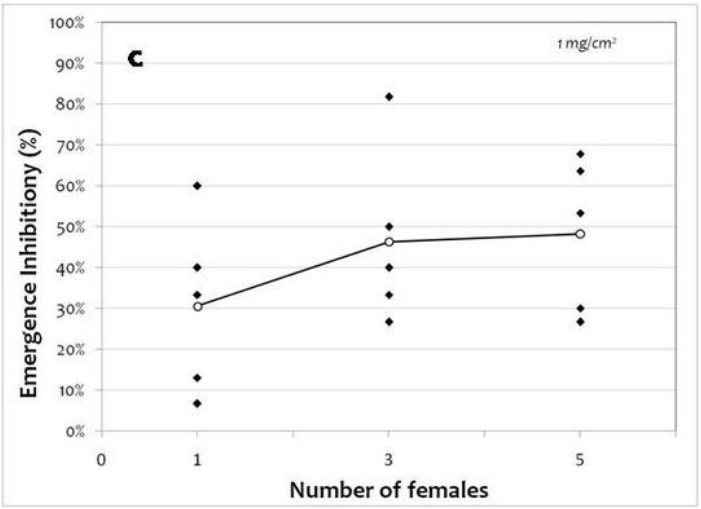


Figure 1

Lufenuron dissemination by gravid Ae. aegypti females to larval breeding sites measured as Emergence Inhibition (%) Legend: Emergence Inhibition (%) achieved in larval microcosms through horizontal movement of lufenuron by gravid Ae. aegypti females that were forced to contact lufenuron-treated paper: 0.2 (A), 0.4 (B) or 1 (C) mg/cm2. Diamonds (♦) data points, circles (●) means of five replicates.